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Gene expression profiling in facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of inherited muscle disease following Duchenne and myotonic dystrophy. FSHD is initially characterized by an asymmetric, progressive weakness of the facial and pectoral girdle muscles. The disorder is inherited in an autosomal dominant fashion with nearly complete penetrance. FSHD results from a deletion of integral copies of a 3.3-kb tandem repeat unit on the long arm of chromosome 4 (D4Z4). A "short" EcoR1 fragment (< 35 kb) containing D4Z4 segregates with the disease, whereas the size of this polymorphic locus in the normal population ranges between 35 and 300 kb. The immediate proximity of the 3.3-kb tandem repeat to the telomere and its sequence similarity to constituitive heterochromatin suggest that this repeat, deleted in FSHD, lies in telomeric heterochromatin. This conclusion is further supported by the fact that, despite intense efforts over the past seven years, there have been no protein coding transcripts identified fr om this repeat sequence. Integral deletions of the heterochromatic D4Z4 repeat instead appear to disrupt the normal expression of adjacent genes, a phenomenon akin to position effect variegation in Drosophila and telomere silencing in yeast. The genes affected by the D4Z4 polymorphism have not been identified, as the 4qter region is rich in repetitive and pseudogene sequences. We are currently using the Affymetrix GeneChip system to examine large-scale differential gene expression in FSHD muscle tissue and myoblast cell lines. In addition, we have used the GeneChip system to examine the regulatory mechanism disrupted by deletions of the heterochromatic repeat D4Z4. Differential gene expression in yeast strains carrying telomeric half-YACs encompassing the FSHD region have been analysed in an effort to identify yeast homologues of genes involved in heterochromatin formation at the D4Z4 locus. A number of genes involved in DNA interstrand crosslink repair were found to be upregulated, perh aps unveiling the initial mutational mechanism in this disease. Thus, we will likely yield immense insight into both the regulatory and mutational mechanisms of the human disease FSHD as well as elucidate the primary cause of this disorder through microarray analysis.

Workman, Paul

Array technology in the molecular pharmacology of anticancer agents

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Exposure of cells to anticancer agents can result in altered gene expression. In general, only a single gene or a small number of genes are studied. To improve the efficiency and value of such experiments, we are interested in using DNA array technology to identify, on a genome-wide scale, drug-responsive genes which might serve both as surrogate markers for drug action and as future targets for drug development. As a means to establish this approach in our Centre we have carried out two exploratory projects. In the first project, we examined gene expression in p53 wild-type and mutant ovarian cell lines established from two patients with ovarian cancer who achieved a complete remission following treatment. Neither line can grow *in vitro* and were passaged as subcutaneous xenografts in nude mice. In the xenograft model both tumours were curable following multiple dosing with cisplatin. Growth-delay experiments following a single dose of 6 mg/kg cisplatin demonstrated that the p53 mutant PXN65 line was more sensitive to cisplatin than the wild-type PXN100 line. Northern blot, RNase protection and western blot demonstrated the induction of p21, MDM-2 and Bax expression in PXN100, but not PXN65. This was consistent with the p53 status of these tumours. These studies were extended to two commercial cDNA arrays, one encompassing genes involved in signal transduction, cell cycle and apoptosis regulation and the other with ESTs of known identity. There was very little difference in the overall constitutive expression pattern between the two cell lines. Following cisplatin treatment the expression of a number of p53-responsive genes was increased in the wild-type PXN100 cell line. There were also changes in the expression of a number of cell-cycle regulators and regulators of the insulin-like growth/survival factor pathway. In contrast, no major changes in gene expression were detected in the p53 mutant PXN65 line. In the second project, we have looked at the effects of a novel agent, the benzoquinone ansamycin 17-allylamino geldanamycin (17-AAG), in two colon adenocarcinoma cancer lines *in vitro*. This agent inhibits hsp-90 activity, a protein required to maintain the stability of a number of signalling proteins, including c-Raf-1, and is about to enter clinical trial in our centre and elsewhere. Treatment of HT29 and HCT116 cells rapidly reduced the levels of c-Raf-1 detected by western blot and inhibited the high levels of constitutive MAP kinase phosphorylation detected in HCT116 cells. This occurred as early as 24 hours and resulted in death by apoptosis 72 hours after treatment. Data detailing changes in gene expression in response to 17-AAG treatment of both HT29 and HCT 116 cells will be presented.

Xiang, Charlie

Comparison of cellular gene expression in Ebola-Zaire and Ebola-Reston virus-infected primary human monocytes

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Ebola viruses are filamentous, enveloped, nonsegmented RNA viruses. Although most Ebola viruses, notably Ebola-Zaire virus, are highly infectious for primates and can cause severe haemorrhagic diseases, Ebola-Reston virus does not cause serious disease in humans. Microarray technology was employed to compare cellular gene responses to Ebola-Zaire and Ebola-Reston virus infection of primary human monocytes, the early targets of Ebola-Zaire virus infection. We found that approximately 200 of 1,400 human genes on the array exhibited changes in expression in response to Ebola-Zaire virus infection after 24 hours. Most affected genes were upregulated in their level of expression, including cytokine and chemokine genes (IL-1, IL-1, IL-6, IL-8, IL-15, MIP-1, MIP-1 and TNF), genes involved in regulation of cell cycle or apoptosis and other genes involved in signal transduction. The gene expression profile from Ebola-Reston–infected monocytes was totally different from that observed with Ebola-Zaire virus. The results from northern-blot or ribonuclease protection assays confirmed the array data. The possible influence of differences in cellular gene expression observed between Ebola-Zaire and Ebola-Reston viruses on the ability of these viruses to cause diseases will be discussed.